Effect of Stem and Root Disease on the Content of Monoterpenes of the Xylem Resin and Phloem Carbohydrate and Nitrogen Fractions of Lodgepole Pine

by John d. Hodges

FINAL REPORT FOR RESEARCH JOINT VENTURE AGMT
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MISSISSIPPI STATE UNIVERSITY
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INTRODUCTION

Forest pests, principally insects, cause millions of dollars in loss to the economy annually. Much research effort has been expended on these pests, but most of the research has been fragmented in that it has dealt with specific issues such as insect biology, sampling, population dynamics, stand hazard rating, or direct control measures. These research efforts have added immensely to our understanding of the pests and the hosts, but in no case have we progressed to the point where we have really effective means for controlling or keeping the pest in check. The major reason why we have not been more successful is that for most pests, and especially the insects, we still do not understand the very basic interactions which take place between the pest and the host tree. Such basic information is essential for effective pest management.

The mechanisms controlling susceptibility to mountain pine beetle attack are associated, in part, with the host's resin system. It is essential that we understand the repertoire of the host defenses, the magnitude and expression of which are contingent on the genotypic and phenotypic "vigor" of the individuals in the host population. Host tree vigor in turn influences the microorganism component, as well as the success of the bark beetle population (Nebeker et al. 1984). This area should continue to receive considerable attention.

From experience and review of the literature (Blanche et al. 1983) an hypothesis (Hodges et al. 1985) has been developed that suggests the importance of the secretory system of pine. Additional information concerning this system is essential, in light of our knowledge concerning the mountain pine beetle (Dendroctonus ponderosae Hopkins) population processes such as host selection,

attack, colonization, and subsequent survival. The parameters discussed below are critical in furthering our understanding of the mountain pine beetle/host interactions.

The physical characteristics and chemical composition of xylem oleoresin are key components in the defensive mechanism(s) of pine trees to bark beetle attack (Schmitt et al. 1988). Oleoresin is a product of living parenchyma cells, and hence is traceable to the original products of photosynthesis for its ultimate chemical derivation. There is evidence implicating acetate as the mobilizable precursor to the site of monoterpene synthesis; thus, factors affecting photosynthesis and the respiratory pathways affect resin synthesis. Loblolly pine xylem oleoresin, for example, consists mainly of resin acids (50 to 85%), and monoterpenes (15 to 45%). Levopimaric acid and palustric acid contribute about one-third of the total resin acids, while α-pinene and β-pinene constitute 87% of the total monoterpenes (Blanche et al. 1983). Levels of monoterpenes and resin acids are affected by the environment, site conditions, mechanical injury, water stress, logging damage, tree age, season, and sampling height. Stress, in general, causes an increase in monoterpenes and a decrease in resin acids. Lightning strikes cause an immediate decline in β-pinene and eventual increase in α-pinene (Blanche et al. 1985).

Monoterpenes are primary elements of resin and intensive research has suggested an attractiveness of these compounds to bark beetles. Limonene has been observed to be toxic to the southern pine beetle, D. frontalis Zimmermann (Coyne and Lott 1976; Hodges et al. 1979), and the western pine beetle, D. brevicomis Leconte (Smith 1965, 1966, 1975; Sturgeon 1979). Myrcene enhances the response of the female western pine beetle to host trees (Bedard et al. 1969; Silverstein 1970). α-pinene has been observed to enhance the attractiveness of host trees to the southern pine beetle (Renwick and Vite 1969). Myrcene and α-pinene have also been documented as beetle pheromone precursors (Brand et al. 1975, 1976; Hughs 1974, 1975). Significantly higher amounts of these two monoterpenes were observed in older age classes of loblolly pine (Schmitt et al 1988) which may partially explain why the southern pine beetle generally attacks the older age classes. Variation in monoterpene concentration and composition has also been observed (Rockwood 1973; Blight and McDonald 1964; Roberts 1970; Smith 1964, 1968, 1977). Also monoterpene composition may change as trees mature (Squillace 1976).

From the literature we might conclude that the "ideal" tree, from a forest manager's point of view, is one that is vigorously growing, with a straight bole and a medium-sized crown. It would exude a lot of resin when attacked by a bark beetle. The resin would be relatively viscous and would flow for a long time. Chemically, its resin would contain low monoterpene, high resin acid levels, and have a low α-pinene

content. This tree would contain an adequate level of defensive chemicals ranging from toxic, low-molecular-weight compounds, to high-molecular-weight digestibility-reducing compounds. It would produce more of the same chemicals when exposed to insect and microorganism attack (Nebeker et al. 1984).

We are interested in comparing the resin composition and nutrient status in lodgepole pine with respect to aspect and disease status. Information concerning these parameters will extend our data base for a comparative basis with other pines.

OBJECTIVES

The objective is to determine if the monoterpene, sesquiterpene, and oxygenated monoterpene content of lodgepole pine xylem resin, phloem nitrogen and carbohydrate levels of trees infected with selected stem and root rots differs from that present in apparently healthy trees.

STUDY AREA

The area selected for this study was located on the north slope of the Uinta Mountains in northeastern Utah at an elevation of 8500 feet (2600 m). The area was between the Hayden Fork and Stillwater Fork of the Bear River some 2.5 miles south of the Bear River Ranger Station which is approximately 30 miles south of Evanston, Wyoming. The area consisted of primarily lodgepole pine with scattered quaking aspen (Populus tremuloides).

Lodgepole pine selected for study averaged DBH of 9.4" (23.88 cm), 57.4' (19.56 m) in height, and were 95 years of age. Within the area the diseases of interest were also present. These included Armillaria root rot AM) (Armillaria mellea (Vahl. ex. Fr.) Kummer, sensu lato, lodgepole pine dwarf mistletoe (DMT) (Arceuthobium americanum Nutt. ex Engelm.) and Comandra blister rust (CBR) (Cronartium comandrae Pk.)

METHODS AND MATERIALS

Resin Composition. Resin from lodgepole pine was collected as described by Nebeker et al. (MS #1). As soon as approximately 1 ml of resin had accumulated in the pipette it was collected and placed in a 1-dram vial, evacuated with nitrogen, and placed on dry ice. The samples, upon returning to the laboratory, were placed in freezers until the monoterpene analysis was conducted. Resin samples were obtained with respect to aspect and disease status (DMT, CBR, AM or CK).

The monoterpenes, sesquiterpenes, and oxygenated monoterpenes were analyzed as outlined by Blanche (1985) using a 5880A Hewlett Packard gas chromatograph equipped with a flame ionization detector. A 8-ft (2.44 m) glass column with an internal diameter of 2 mm, packed with 10% Carbowax 20M on 80 to 100 mesh acid-washed Chromosorb W. Samples were run under the following conditions: N_2 flow rate = 20 ml/min.; H_2 flow rate = 40 ml/min.; air flow rate = 250 ml/min.; detector temperature = 200 °C; injection port temperature = 225 °C; oven temperature was programmed with initial temperature of 60 °C for 5 minutes, increasing at the rate of 5 degrees C per minute to the final temperature of 130 °C and remained constant at that temperature for 12 minutes. Identities of the compounds were verified co-chromatographically using authentic terpenes. Quantities of terpenes are expressed in mg/100mg of oleoresin using terpinene-4-ol as the internal standard.

Nutrient Analysis. After resin flow and collection an area at breast height, on each aspect (North and South), had the outer bark removed with a draw knife exposing the primary phloem. A sample of the primary phloem was removed, placed in aluminum foil, labelled, and then placed on dry ice in a cooler. The samples were approximately 3 inches^2 (20 cm^2). Samples were returned to the laboratory and placed in freezers until the laboratory analysis could be conducted. Prior to analysis the samples were removed from the freezer an ground in liquid nitrogen using mortar and pestle, and lyophilized. The lyophilized samples were stored in dessicators at minus 16°C until chemically analyzed.

Total nitrogen was determined through nesslerization using 10 mg of freeze-dried sample. The protocol consisted of digesting the sample in 20% sulfuric acid and heating until completely charred, then clearing it with 30% reagent grade hydrogen peroxide. The cleared solution was neutralized with 4N KOH prior to standard nesslierization. Absorbance was read at 490 nm. Quantification was based on a standard curve developed using ammonium sulfate.

Determination of total sugar (reducing and non-reducing sugars) was based on the Somogyi-Nelson procedure (Hodge and Hofreiter 1961). This procedure was slightly modified in that polyvinylpholypyrrolidone (PVPP) was used to clean the extract. Boiling 80% ethanol was used to extract the sugars from 50 mg samples. The extract was filtered with white ribbon filter paper #589 (Schleicher and Schuell, Inc.). The residue was recovered and saved for starch analysis. The filtrate was evaporated to near dryness, resuspended in 10 ml distilled water, stirred in 200 mg PVPP, further filtered with blue ribbon filter paper # 589 and made to volume. An aliquot was assayed colorimetrically for reducing power at 500 nm. Non-reducing sugar was determined through invertase digestion from a portion of the filtrate used for reducing

sugar determination and then assayed for reducing power as described above. Starch was analyzed from the residue saved after ethanol extraction. The residue was hydrolyzed by boiling in 0.2N sulfuric acid for 1 hour, filtered, and then assayed colorimetrically for reducing sugars. Details of the procedure are found in Smith et al. (1964). Accuracy of the prodedure was checked using the phenol sulfuric acid method of Dubois et al. (1956).

The amino nitrogen fraction was determined from a portion of the reducing sugar extract (Rosen 1957). After color reaction with ninhydrin, the solution was diluted with isopropyl alcohol-water (1:1 v/v) and colorimetrically read at 570 nm. Quantification was based on a standard curve developed using aspartic acid.

Analysis. Data analysis consisted of appropriate transformations with respect to the analysis performed and units of measure. The data were analyzed using SPSS V4.0 procedures (SPSS Inc. 1990) for ANOVA and Multiple Range testing (LSD Procedure). Level of significance was set at $p = 0.05$.

RESULTS

The basic descriptors of the trees is contained in the first portion of Table 1. There were no significant differences in the ages of the trees sample. There were differences in diameter and height with the CK's being the tallest and largest in diameter. This being attributed to the effects of the particular disease. Significantly more resin was collected from the DMT trees than the CK's (apparently healthy trees).

Resin Composition. Through gas chromatography, we detected the following volatiles in lodgepole pine stem oleoresin: monoterpenes, oxygenated terpenes, a sesquiterpene (longifolene), and a phenylpropanoid (4-allylanisole). Table one contains the aspect comparisons. Table 2 combines aspect to increase the sample size for a closer look at the differences between four classes of trees. Since only camphene was found to be significantly different with respect to aspect the following results will be direct at the results presented in Table 2.

The monterpene α-thujene, reported present in lodgepole pine growing in northeastern Oregon (Raffa and Berryman 1982) was not detected in any of our samples. Also, Shrimpton (1973) did not find this monoterpene from his sample trees in British Columbia. Under the chromatographic conditions used, we were not able to detect limonene despite deliberate efforts in optimizing chromatographic conditions. The problem involved was the high concentration of β-phellandrene which masked the limonene peak. We observed this when running the limonene and β-phellandrene standards whereby the two would separately distinctly (retention times= 11.7 and

12.25, respectively) both at low concentrations. However, when the proportion of β-phellandrene was increased to a level approximating its level in the oleoresin, the limonene peak fused into the β-phellandrene peak thus precluding the detection and quantification of limonene and resulting in the overestimation of β-phellandrene.

Of the 12 monoterpenes found present in the oleoresin, three monoterpenes (tricyclene, α-pinene, and camphene) were generally lower in concentrations in check trees than in the diseased trees, and one monoterpene (myrcene) was higher in concentration in the check trees than in the diseased trees (Table 3). The concentration of the other monoterpenes did not vary between the check and diseased trees.

Two oxygenated terpenes were found to be significantly different in concentrations between check and diseased trees (Table 3). The camphor content was higher in check trees than the diseased trees whereas α-terpineol content was lower in check trees than in diseased trees. The level of the sesquiterpene longifolene was no different in the check trees from the diseased ones. On the other hand, the phenylpropanoid 4-allylanisole was significantly higher in check trees than in diseased trees.

The terpenes (especially the monoterpenes) have been implicated as host resistance factors as well as bark beetle attractants. Depending on the pest species or kind of disease organisms, each monoterpene may vary in its inhibitory capacity. Toxicity of the monoterpene components of southern pine oleoresin to the southern pine beetle has been reported (Coyne and Lott 1976). Smith (1963) has, likewise, demonstrated the toxic properties of pine resin vapors to other species of Dendroctonus. More importantly the antibiotic or inhibitory properties of monoterpenes on the different species of bark beetle associated fungi have been demonstrated (Bridges 1987, Cobb et al. 1968, Raffa et al. 1985). Cobb et al. (1968) showed that myrcene was the most inhibitory to four species of Ophistoma, whereas Bridges (1987) found that 4-allylanisole was the most inhibitory to all three symbiotic fungi associated with the southern pine beetle. α-pinene has been implicated as bark beetle attractant and is known to synergize with insect produced pheromone. In another way, α-pinene has been hypothesized to be the most important monoterpene in the beetle's perception of a resistant host (Bordash and Berryman 1977). Little is known about α-terpineol in relation to bark beetle associated fungi but it has been known to serve as an attractant of some European bark beetles. Although these specific properties of individual monterpenes are clearcut, their functions when in combination with the other component monoterpenes, have not been adequately addressed.

Phloem Chemistry. No significant differences were detected with respect to aspect (Table 1). The CK trees have significantly higher starch, total nitrogen, and free amino-N contents than the diseased trees (Table 3). The CBR trees contained significantly lower reducing and nonreducing sugars than the CK, DMT, and AM trees do (Table 3).

These major compounds present in the phloem play two major roles in host-bark beetle-micoorganism interactions: first, as substrates for host physiology, and second as substrates for bark beetle-microorganisms' growth and survival. Thus, levels of these compounds represent host defensive potential and host suitability for bark beetle brood development. The much lower levels of these compounds in the diseased trees than in the healthy trees imply that portions of these substrates are being channelled into the disease organisms, hence, constituting an energy drain that otherwise would be used for growth by the host trees. Because the sugars (reducing and nonreducing) are two very dynamic metabolic pools, subject to rapid interconversions as conditions change, their levels at any given time may not reflect the overall value of these compounds in terms of host resistance. Similar results have likewise been reported for ponderosa pine (Miller et al. 1968) and shortleaf pine (Hepting 1945) whereby the diseased trees had lower carbohydrate contents than the healthy ones. On the other hand, Reid and Gates (1972) found no significant differences in starch concentration between resistant and nonresistant lodgepole pine trees. This may be attributed to the lower sensitivity of the technique for their starch analysis.

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Table 1. Summary of lodgepole pine components with respect to aspect and associated diseases on the north slope of the Uinta Mountains, south of Evanston, WY, 1989.

Component	DMT	CBR	AM	CK
Age (yrs)	100.3a	95.2a	97.6a	95.0a
DBH (cm)	24.4a	22.4b	23.9ab	24.6a
Height (m)	17.6b	15.9c	17.6b	19.0a
Radial Growth (mm/last5 yrs)	3.4ab	2.9b	3.6ab	4.7a
Radial Growth (mm/last10 yrs)	6.1b	5.7b	6.3b	8.8a
Resin Flow				
North Aspect (ml/2 hrs)	0.60a	0.46ab	0.33ab	0.20b
South Aspect (ml/2 hrs)	0.46ab	0.42ab	0.59a	0.11b
North Aspect (ml/24 hrs)	2.37a	1.75a	1.58a	1.28a
South Aspect (ml/24 hrs)	1.89a	1.69a	1.96a	0.96b
N+S (ml/2 hrs)	1.05a	0.82ab	1.02ab	0.31b
N+S (ml/24 hrs)	4.26a	3.24ab	3.79ab	2.24b
Tricyclene (mg/100 mg)				
North Aspect	0.017a	0.013a	0.019a	0.007b
South Aspect	0.016a	0.010bc	0.015ab	0.004c
α–Pinene (mg/100 mg)				
North Aspect	0.729a	0.695a	0.592a	0.683a
South Aspect	0.763a	0.671ab	0.709ab	0.527b
β–Pinene (mg/100 mg)				
North Aspect	2.32a	2.59a	2.31a	0.57b
South Aspect	2.25a	2.59a	1.93a	1.78a
Camphene (mg/100 mg)				
North Aspect	0.142a	0.132a	0.124a	0.127a*
South Aspect	0.145a	0.121ab	0.139a	0.093b*
Sabinene (mg/100 mg)				
North Aspect	0.375a	0.350a	0.516a	0.386a
South Aspect	0.636a	0.349a	0.466a	0.329a
Δ–3–Carene (mg/100 mg)				
North Aspect	4.64a	5.31a	5.68a	4.80a
South Aspect	4.29a	6.27a	6.53a	4.68a
Myrcene (mg/100 mg)				
North Aspect	0.975ab	0.907b	0.948ab	1.480a
South Aspect	0.982a	0.980a	1.020a	1.070a
α-Terpinene (mg/100 mg)				
North Aspect	0.001b	0.010b	0.040a	0.011b
South Aspect	0.012a	0.027a	0.016a	0.022a
β–Phellandrene (mg/100 mg)				
North Aspect	17.26a	17.21a	17.01a	16.58a
South Aspect	17.69a	15.32ab	16.14ab	14.46b
γ–Terpinene (mg/100 mg)				
North Aspect	0.084b	0.110b	0.232a	0.078b
South Aspect	0.083ab	0.113a	0.101a	0.050b
p-Cymene (mg/100 mg)				
North Aspect	0.011a	0.015a	0.009a	0.008a
South Aspect	0.013a	0.007a	0.009a	0.005a

Table 1. Summary of lodgepole pine components with respect to aspect and associated diseases on the north slope of the Uinta Mountains, south of Evanston, WY, 1989 (continued).

Component	DMT	CBR	AM	CK
Terpinolene (mg/100 mg)				
North Aspect	0.464a	0.532a	0.422a	0.487a
South Aspect	0.441a	0.647a	0.620a	0.496a
Camphor (mg/100 mg)				
North Aspect	0.011b	0.011b	0.024ab	0.053a
South Aspect	0.011b	0.0278ab	0.015ab	0.038a
Linalool (mg/100 mg)				
North Aspect	0.017b	0.015b	0.057a	0.023b
South Aspect	0.016a	0.021a	0.028a	0.052a
Longifolene (mg/100 mg)				
North Aspect	0.001a	0.009a	0.000a	0.004a
South Aspect	0.001a	0.006a	0.000a	0.006a
Bornyl Acetate (mg/100 mg)				
North Aspect	0.000b	0.000b	0.081a	0.001b
South Aspect	0.000b	0.000b	0.015a	0.004b
4-Allylanisole (mg/100 mg)				
North Aspect	0.235ab	0.178ab	0.120b	0.319a
South Aspect	0.251a	0.158ab	0.102b	0.271a
α-Terpineol (mg/100 mg)				
North Aspect	0.059a	0.048ab	0.043ab	0.016b
South Aspect	0.069a	0.047ab	0.0412ab	0.011b
Nitrogen (µg/100 mg)				
North Aspect	747.88b	812.16b	932.47ab	1011.54a
South Aspect	892.25a	807.58a	961.38a	934.85a
Amino Nitrogen (µg/100 mg)				
North Aspect	78.69b	75.65b	77.66b	85.50a
South Aspect	77.61b	76.09b	76.72b	85.05a
Sugars (mg/100 mg)				
Reducing				
North Aspect	3.10ab	2.61b	2.80b	3.58a
South Aspect	3.16ab	2.63b	3.16ab	3.66a
Non-Reducing				
North Aspect	3.24a	2.12b	3.11ab	3.74a
South Aspect	3.20ab	2.31b	3.55a	4.21a
Starch (mg/100 mg)				
North Aspect	21.48b	21.86ab	22.07ab	23.40a
South Aspect	21.89b	21.48b	22.20b	24.01a

DMT = Dwarf Mistletoe, (Arceuthobium americanum Nutt. ex Engelm.)

CBR = Commander Blister Rust, (Cronartium comandrae Pk.)

AM = Armelleria Root Rot, (Armillaria mellea (Vahl. ex. Fr.) Kummer,sensu lato

CK = Checks or controls, they appeared to be "disease free."

Values within a row, with the same letter(s), are not significantly different ($p>0.05$).

Values followed with an * are significantly different with respect to aspect for said component ($p<0.05$)

Table 2. Summary of selected lodgepole pine resin components with respect to associated diseases on the north slope of the Uinta Mountains, south of Evanston, WY, 1989.

Component	DMT	CBR	AM	CK
Resin Flow				
ml/2 hrs	0.47a	0.44a	0.53a	0.15b
ml/24 hrs	1.79ab	1.72ab	2.13a	1.12b
Tricyclene (mg/100 mg)	0.016a	0.011b	0.017a	0.006c
α–Pinene (mg/100 mg)	0.747a	0.684ab	0.664ab	0.614b
β–Pinene (mg/100 mg)	2.289ab	2.598a	1.413b	2.078ab
Camphene (mg/100 mg)	0.144a	0.127ab	0.133a	0.112b
Sabinene (mg/100 mg)	0.512a	0.349a	0.485a	0.361a
Δ–3–Carene (mg/100 mg)	4.458a	5.781a	6.211a	4.752a
Myrcene (mg/100 mg)	0.979b	0.942b	0.994ab	1.302a
α-Terpinene (mg/100 mg)	0.010b	0.018ab	0.025a	0.016ab
β–Phellandrene (mg/100 mg)	17.491a	16.299a	16.477a	15.643a
γ–Terpinene (mg/100 mg)	0.083b	0.111ab	0.151a	0.066b
p-Cymene (mg/100 mg)	0.012a	0.011a	0.009a	0.007a
Terpinolene (mg/100 mg)	0.452a	0.587a	0.544a	0.491a
Camphor (mg/100 mg)	0.011b	0.019b	0.018b	0.046a
Linalool (mg/100 mg)	0.016b	0.018ab	0.039a	0.036ab
Longifolene (mg/100 mg)	0.001b	0.007a	0.000b	0.005ab
Bornyl.Acetate (mg/100 mg)	0.000b	0.000b	0.040a	0.002b
4-Allylanisole (mg/100 mg)	0.243ab	0.168bc	0.109c	0.298a
α-Terpineol (mg/100 mg)	0.064a	0.048a	0.042ab	0.014b

DMT = Dwarf Mistletoe, (Arceuthobium americanum Nutt. ex Engelm.)
CBR = Commander Blister Rust, (Cronartium comandrae Pk.)
AM = Armelleria Root Rot, (Armillaria mellea (Vahl. ex. Fr.) Kummer,sensu lato
CK = Checks or controls, they appeared to be "disease free."
Values within a row, with the same letter(s), are not significantly different ($p>0.05$).

Table 3. Summary of lodgepole pine nutrient components with respect to associated diseases on the north slope of the Uinta Mountains, south of Evanston, WY, 1989.

Component	DMT	CBR	AM	CK
Nitrogen (µg/100 mg)	820.07b	809.75b	949.34ab	974.24a
Amino Nitrogen (µg/100 mg)	78.15b	75.88b	77.11b	85.28a
Sugars (mg/100 mg)				
Reducing	3.13ab	2.62b	3.01b	3.62a
Non-Reducing	3.22a	2.22b	3.39a	3.96a
Starch (mg/100 mg)	21.68b	21.66b	22.15b	23.70a

DMT = Dwarf Mistletoe, (Arceuthobium americanum Nutt. ex Engelm.)

CBR = Commander Blister Rust, (Cronartium comandrae Pk.)

AM = Armelleria Root Rot, (Armillaria mellea (Vahl. ex. Fr.) Kummer,sensu lato

CK = Checks or controls, they appeared to be "disease free."

Values within a row, with the same letter(s), are not significantly different ($p>0.05$).